CHEMICAL CONSTITUENTS AND ANTIOXIDANT ACTIVITIES OF *AQUILARIA MALACCENSIS* LAMK. (AGARWOOD) LEAVES

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SUPERVISOR'S DECLARATION

We hereby declare that We have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Master of Science (Industrial Chemistry).

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STUDENT'S DECLARATION

I hereby declare that the work in this thesis is based on my original work except for quotations and citation which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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ABSTRAK

Spesies *Aquilaria* mengandungi pelbagai metabolit sekunder termasuk pelbagai sebatian fenolik yang dikatakan bertindak sebagai antioksidan yang sangat berkesan. Kajian terhadap sifat fitokimia dan antioksidan spesies *Aquilaria malaccensis* dari keluarga Thymelaeaceae telah dilakukan. Pengekstrakan fitokimia dari daun dilakukan dengan menggunakan kaedah pengekstrakan konvensional; pengekstrakan refluks menggunakan air dan penyerapan dengan metanol yang menghasilkan ekstrak air (WE) dan ekstrak metanol (ME). Pengekstrakan, pemeringkatan dan pemisahan campuran menggunakan beberapa teknik kromatografi (kromatografi kolum dan kromatografi lapisan nipis) telah digunakan dalam proses pemisahan sebatian tulen. Untuk mengenal pasti sebatian yang mudah meruap dalam *A. malaccensis* daun, dua teknik digunakan, iaitu, pengekstrakan mikro fasa pepejal (SPME) dan desorpsi haba langsung (DTD), kemudian dianalisis dengan menggunakan teknik gas kromatografi iaitu, GC-FID dan GC-MS. Sementara itu, kromatografi cecair ultra tinggi dengan kuadrupol spektrometri jisim masa penerbangan (UPLC-QToF / MS) digunakan untuk menentukan sebatian kimia yang tidak mudah meruap di dalam tumbuhan tersebut. Potensi antioksidan daripada ekstrak *A. malaccensis* dikawal oleh kadar perangkap radikal bebas (DPPH) dan pengurangan kapasiti antioksidan melalui tembaga (CUPRAC). Perbezaan signifikasi adalah berdasarkan nilai p di mana $p<0.05$ dianggap ada perbezaan dan sebaliknya. Beberapa komponen yang biasa ditemui adalah asid *n*-heksadekanoik, eudesmol dan oxo-agarospirol. Ekstrak air dan metanol, yang mana mengandungi jumlah kandungan fenolik yang tinggi (191.005 ± 0.002 dan 177.927 ± 0.001 mg dari GAEs/g ekstrak) menunjukkan kuasa penurunan dan menghapuskan aktiviti radikal bebas yang tinggi. Pemisahan ekstrak heksana membawa kepada pengasingan friedelanol dan pemisahan ekstrak diklorometana membawa kepada pengasingan friedelin. Struktur sebatian tulen tersebut dikenalpasti melalui kaedah seperti 1D (^{1}H, ^{13}C, DEPTQ), 2D (COSY, HSQC, HMBC) NMR, MS, UV, FTIR dan juga melalui perbandingan maklumat spektra daripada kajian lepas.

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ABSTRACT

Aquilaria species contains variety of secondary metabolites including various phenolic compounds which have been reported as excellent antioxidants. A study on phytochemical and antioxidant properties of *Aquilaria malaccensis* from Thymelaeaceae family was performed. The extraction of phytochemicals from the leaves were performed using conventional extraction methods: reflux extraction using water and maceration using methanol to obtain water extract (WE) and methanol extract (ME), respectively. Solvent–solvent extraction, fractionation and separation using different chromatographic techniques (column chromatography and thin layer chromatography) were used for isolation of pure compounds. In order to identify the volatile aroma compounds in the leaves of *A. malaccensis*, two methods were used namely solid-phase microextraction (SPME) and direct thermal desorption (DTD), in combination with gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis. The ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QToF/MS) was used for determination of non-volatile chemical compounds present in the leaves. The potential antioxidative activity of the leaves extracts of *A. malaccensis* was evaluated via radical scavenging assay (DPPH) and copper reducing antioxidant capacity (CUPRAC) assays. Significant differences were based on p values where $p<0.05$ were considered significantly different and vice-versa. Some of the commonly identified chemical components were *n*-hexadecanoic acid, eudesmol and oxo-agarospirol. WE and ME extracts with the highest total phenolic contents (191.005 ± 0.002 and 177.927 ± 0.001 mg of GAEs/g extract) showed strong reducing power and scavenging radical activity. Friedelanol and friedelin were isolated from the fractionation of hexane and dichloromethane extracts, respectively. The structure of the isolated compounds was elucidated by using spectroscopic methods namely 1D (^{1}H, ^{13}C, DEPTQ), 2D (COSY, HSQC, HMBC) NMR, MS, UV, FTIR and by comparison with literature values of published data spectra.

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LIST OF SYMBOLS

α	alpha
β	beta
°C	Celsius
cm	centimetre
cm^{-1}	per centimetre
$CuCl_2$	copper (II) chloride
δ	chemical shift in ppm
eV	electron volt
γ	gamma
g	gram
hr	hour
KBr	potassium bromide
kg	kilogram
Kv	kilo volt
λ_{max}	maximum wavelength
μM	micromolar
μ	micro
μL	microlitre
m	metre
mL	millilitre
mm	millimetre
mM	millimolar
μM	micromolar
mg/mL	milligram per millilitre
$MgCl_2$	magnesium chloride
MHz	mega Hertz
m/z	mass to charge ratio
Na	sodium
NH_4OH	ammonium hydroxide
nm	nanometre
%	percentage

LIST OF ABBREVIATION

AA	ascorbic acid
BPI	based peak ion
^{13}C NMR	carbon nuclear magnetic resonance
$CDCl_3$	deuterated chloroform
COSY	correlation spectroscopy
CUPRAC	cupric reducing capacity
dbh	diameter at breast height
DCM	dichloromethane
DEPTQ	distortionless enhancement by polarization transfer with retention of quaternaries
DPPH	2,2-diphenyl-1-picrylhydrazyl
DTD	direct thermal desorption
EIMS	electron impact mass spectrometry
EtOAc	ethyl acetate
EtOH	ethanol
GC-FID	gas chromatography-flame ionization detector
GC-MS	gas chromatography-mass spectrometry
Hx	hexane
HPLC	high performance liquid chromatography
^{1}H NMR	proton nuclear magnetic resonance
HMBC	heteronuclear multiple bond coherence
HSQC	heteronuclear single quantum coherence
EC_{50}	concentration required to obtain a 50 % antioxidant effect
GAE	mg/g gallic acid equivalents
IC_{50}	concentration of drug required to inhibit cell growth by 50 %
IR	infrared
J	coupling constant
M^+	molecular ion
ME	methanol extract
MS	mass spectrum
NIST	National Institute of Standards and Technology

NMR	nuclear magnetic resonance
pH	power of hydrogen
RI	retention index
RP-18	reverse phase silica gel
SPME	solid phase microextraction
t_R	retention time
TIC	total ion current chromatogram
TPC	total phenolic content
TLC	thin layer chromatography
UPLC-QToF/MS	ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry
UV-Vis	ultraviolet-visible spectroscopy
WE	water extract

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